Genome-Wide Meta-Analysis of Psoriatic Arthritis Identifies Susceptibility Locus at *REL*

Eva Ellinghaus[1], Philip E. Stuart[2], David Ellinghaus[1], Rajan P. Nair[2], Sophie Debrus[3], John V. Raelson[4], Majid Belouchi[5], Trilokraj Tejasvi[2], Yanming Li[6], Lam C. Tsoi[6], Anna T. Onken[7], Tonu Esko[8,9,10], Andres Metspalu[8,9,10], Proton Rahman[11], Dafna D. Gladman[12], Anne M. Bowcock[13], Cynthia Helms[13], Gerald G. Krueger[14], Sulev Koks[15], Külli Kingo[16], Christian Gieger[17], H. Erich Wichmann[18,19,20], Ulrich Mrowietz[21], Stephan Weidinger[21], Stefan Schreiber[1,22], Gonçalo R. Abecasis[6], James T. Elder[2,23], Michael Weichenthal[21] and Andre Franke[1]

Psoriatic arthritis (PsA) is a chronic inflammatory musculoskeletal disease affecting up to 30% of psoriasis vulgaris (PsV) cases and approximately 0.25 to 1% of the general population. To identify common susceptibility loci, we performed a meta-analysis of three imputed genome-wide association studies (GWAS) on psoriasis, stratified for PsA. A total of 1,160,703 single-nucleotide polymorphisms (SNPs) were analyzed in the discovery set consisting of 535 PsA cases and 3,432 controls from Germany, the United States, and Canada. We followed up two SNPs in 1,931 PsA cases and 6,785 controls comprising six independent replication panels from Germany, Estonia, the United States, and Canada. In the combined analysis, a genome-wide significant association was detected at 2p16 near the *REL* locus encoding c-Rel (rs13017599, $P = 1.18 \times 10^{-8}$, odds ratio (OR) = 1.27, 95% confidence interval (CI) = 1.18–1.35). The rs13017599 polymorphism is known to associate with rheumatoid arthritis (RA), and another SNP near *REL* (rs702873) was recently implicated in PsV susceptibility. However, conditional analysis indicated that rs13017599, rather than rs702873, accounts for the PsA association at *REL*. We hypothesize that c-Rel, as a member of the Rel/NF-κB family, is associated with PsA in the context of disease pathways that involve other identified PsA and PsV susceptibility genes including *TNIP1*, *TNFAIP3*, and *NFκBIA*.

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[1]*Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Kiel, Germany;* [2]*Department of Dermatology, University of Michigan, Ann Arbor, Michigan, USA;* [3]*Gatineau, Quebec, Canada;* [4]*PGX-Services, Montreal, Quebec, Canada;* [5]*Genizon BioSciences, St Laurent, Quebec, Canada;* [6]*Department of Biostatistics, Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan, USA;* [7]*Department of Dermatology and Allergy, Technische Universität München, Munich, Germany;* [8]*Estonian Genome Center and Center of Translational Genomics, University of Tartu, Tartu, Estonia;* [9]*Estonian Biocenter, Tartu, Estonia;* [10]*Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia;* [11]*Department of Medicine, Memorial University, St John's, Newfoundland, Canada;* [12]*Division of Rheumatology, University of Toronto, Psoriatic Arthritis Program, University Health Network, Toronto, Ontario, Canada;* [13]*Department of Genetics, Washington University School of Medicine, St Louis, Missouri, USA;* [14]*Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah, USA;* [15]*Department of Physiology, University of Tartu, Tartu, Estonia;* [16]*Department of Dermatology and Venerology, University of Tartu, Tartu, Estonia;* [17]*Institute of Genetic Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany;* [18]*Institute of Epidemiology I, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany;* [19]*Institute of Medical Informatics, Biometry and Epidemiology, Ludwig-Maximilians University, Munich, Germany;* [20]*Klinikum Grosshadern, Munich, Germany;* [21]*Department of Dermatology, University Hospital Schleswig-Holstein, Christian-Albrechts-University, Kiel, Germany;* [22]*Popgen Biobank, University Hospital Schleswig-Holstein, Christian-Albrechts-University, Kiel, Germany and* [23]*Ann Arbor Veterans Affairs Hospital, Ann Arbor, Michigan, USA*

Correspondence: Eva Ellinghaus, Institute of Clinical Molecular Biology, Christian-Albrechts-University Kiel, Schittenhelmstrasse 12, D-24105 Kiel, Germany. E-mail: e.ellinghaus@ikmb.uni-kiel.de

Abbreviations: CI, confidence interval; GWAS, genome-wide association study; PsA, psoriatic arthritis; PsV, psoriasis vulgaris; SNP, single-nucleotide polymorphism

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INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory musculoskeletal disease that occurs in the context of psoriasis vulgaris (PsV) affecting up to 30% of psoriasis patients (Gladman *et al.*, 2005). Apart from the skin manifestation of PsV, the clinical phenotype of PsA overlaps with ankylosing spondylitis and rheumatoid arthritis (RA) (Castelino and Barton, 2010). Although PsV has a prevalence of up to 3% in populations of European ancestry (Bowcock and Barker, 2003; Griffiths and Barker, 2007), the estimated prevalence of PsA varies from 0.25 to 1% (Gladman *et al.*, 2005). The strong genetic component of PsA is evidenced by the high sibling recurrence risk (λ_S) of 27–47 (Gladman *et al.*, 2003; Myers *et al.*, 2005; Chandran *et al.*, 2009), which is much higher than the estimated $\lambda_S = 4$–11 of PsV (Elder *et al.*, 1994; Bhalerao and Bowcock, 1998), suggesting that PsA patients harbor additional susceptibility loci beside the loci that contribute to risk for PsV. In addition to the significant heritable component, environmental risk factors have an important role for both PsV and PsA. The onset of PsA generally occurs in the fourth and fifth decades of life. In nearly 80% of patients, the skin disease (PsV) precedes the arthritis (PsA). The clarification of similarities and differences of the genetic background in PsV and PsA offers chances for well-targeted interventions, i.e., specific therapies for both conditions. It would also provide an opportunity to identify PsV patients with increased risk of PsA and would allow

treatment at the earliest signs of joint involvement (Bowes and Barton, 2010).

Several susceptibility loci for PsV have been identified with genome-wide levels of statistical significance in populations of European origin, including *HLA-Cw6, IL12B, IL23R, IL4-IL13, IL23A, TNIP1, TNFAIP3, LCE3B-LCE3C, RNF114, TRAF3IP2, NFκBIA, NOS2, FBXL19, TYK2, IFIH1, REL, IL28RA,* and *ERAP1* (Liu *et al.*, 2008; Duffin *et al.*, 2010; Ellinghaus *et al.*, 2010; Strange *et al.*, 2010; Stuart *et al.*, 2010). Many of the susceptibility loci for PsV tested so far are also genome-wide significantly associated with PsA such as *HLA-Cw6, IL12B, TNIP1, FBXL19,* and *TRAF3IP2* or at the level of $P<0.05$ such as *IL23R, IL23A, TNFAIP3, NFκBIA, NOS2,* and *IL13* (Bowes and Barton, 2010; Ellinghaus *et al.*, 2010; Huffmeier *et al.*, 2010; Stuart *et al.*, 2010; Eder *et al.*, 2011). However, because the causative allele(s) have not been identified within most of these regions, their actual contribution to disease risk remains unknown.

To further our understanding of PsA etiology, we conducted a meta-analysis of the PsA subsets of three independent genome-wide association studies (GWAS) of PsV from Kiel, Germany (Ellinghaus *et al.*, 2010), the Collaborative Association Study of Psoriasis (CASP; Nair *et al.*, 2009) and Canada (Genizon, unpublished data), consisting of 535 PsA patients and 3,432 healthy controls. Genotype imputation with HapMap phase 3 reference samples considerably increased the genomic coverage of the three GWAS data sets and facilitated the combined analysis. For replication, we selected 10 single nucleotide polymorphisms (SNPs) based on their *P*-value ranking in the meta-analysis and genotyped them in two independent replication sets from Germany and Estonia. The two most strongly associated SNPs were further genotyped in four additional replication cohorts from Michigan, the National Psoriasis Foundation, the CASP Deep Follow-up (CASP-DFU), and Estonia. Together, this replication set comprised 1,931 PsA cases and 6,785 controls (Supplementary Table S1 online). We identified genome-wide significant association for SNP rs13017599, which is located between the genes *REL* and *PUS10*, and has been already reported to be associated with RA (Gregersen *et al.*, 2009). Our results indicate that *REL* is an important candidate susceptibility locus not only for PsV and RA but also for PsA.

RESULTS

Before genotype imputation and meta-analysis, we applied extensive quality control filters to the three GWAS data sets separately. We excluded all samples with low genotyping rate (<95%), as well as non-European outliers or cryptically related individuals. Further, we excluded SNPs with a minor allele frequency <1%, a genotyping success rate <95%, or deviation of the genotype distribution from Hardy–Weinberg equilibrium (HWE) in the controls ($P<10^{-4}$). After applying these stringent quality control filters, genotype imputation was performed with Beagle v.3.2.1 (Browning and Browning, 2009) using HapMap phase 3 reference haplotypes (Altshuler *et al.*, 2010). Only SNPs imputed with high confidence (estimated r^2 between imputed and true genotypes $\geqslant 0.3$) in all three GWAS were considered for subsequent statistical analysis, leaving a total of 1,160,703 SNPs. A logistic regression procedure was applied to test both genotyped and imputed SNPs for association. To account for uncertainty in the imputation procedure, we used allele dosages from the imputation. After combining the association results of all three scans, a low genomic-control value (meta GWAS: $\lambda_{GC}=1.02$) indicated a minimal overall inflation of the test statistics due to population stratification (Supplementary Figure S1 online). Our combined discovery panel had 80% power to detect variants conferring odds ratios (ORs) of 1.40 or higher at the 5% significance level, assuming a frequency of the disease-associated risk allele of at least 15% in controls (Supplementary Figure S2 online). In line with previous studies, we detected the strongest associations with PsA at SNPs in the HLA complex at chromosome 6p21 approximately 35 kb upstream of *HLA-C* (rs12212594, $P=4.98\times10^{-38}$, OR = 3.60; rs12191877, $P=3.95\times10^{-35}$, OR = 2.73; and rs10484554, $P=3.03\times10^{-34}$, OR = 2.72; Manhattan plot see Figure 1). SNP rs12191877 was previously identified as most strongly associated with PsV in a GWAS of individuals with genuine European ancestry (Nair *et al.*, 2009), whereas rs10484554 has been described to be most significantly associated in a combined study of PsA and PsV (Liu *et al.*, 2008). In our discovery panel, rs12212594 was in moderate linkage disequilibrium with rs12191877 ($r^2=0.57$), indicating that these signals are not independent. In addition, we found evidence of association at the previously reported susceptibility loci *TNIP1* (Nair *et al.*, 2009; rs17728338, $P=6.75\times10^{-10}$, OR = 2.07) and *IL12B* (Liu *et al.*, 2008; rs3212227, $P=3.82\times10^{-7}$, OR = 1.64). For selection of follow-up SNPs, we excluded all SNPs within the extended major histocompatibility complex region (7,773 SNPs on chromosome 6 at 25–34 Mb), as well as SNPs within the genes *TNIP1* and *IL12B*, as these loci have already been shown to be associated with PsA at a genome-wide significant level. Furthermore, we used only those SNPs that were available in all three scans, and that showed no heterogeneity between the studies (I^2 statistic = 0). We selected 10 SNPs for replication (see Manhattan plot, Figure 1) in two independent samples from Germany and Estonia (replication panels 1 and 2) comprising 252 PsA cases and 1,740 controls. All SNPs passed quality control measures, which means that they had a high call rate (>95% in cases or controls), were not monomorphic (minor allele frequency >1% in cases or controls), and did not deviate from the HWE in the control population (*P*-value $>10^{-4}$). Taking into account the minor differing genetic backgrounds of the two replication study groups, we used the Cochran–Mantel–Haenszel test for the combined analysis (Table 1). Of the 10 follow-up SNPs, 2 SNPs were nominally associated with PsA in the replication data set (rs13017599 and rs2829866 with $P<0.05$) and were therefore genotyped in four additional replication panels from Michigan, the National Psoriasis Foundation, the CASP-DFU, and Estonia (replication panels 3 through 6), yielding a replication sample consisting of 1,931 PsA cases and 6,785 controls. In the combined replication analysis, rs13017599—located 15 kb downstream of *REL* and

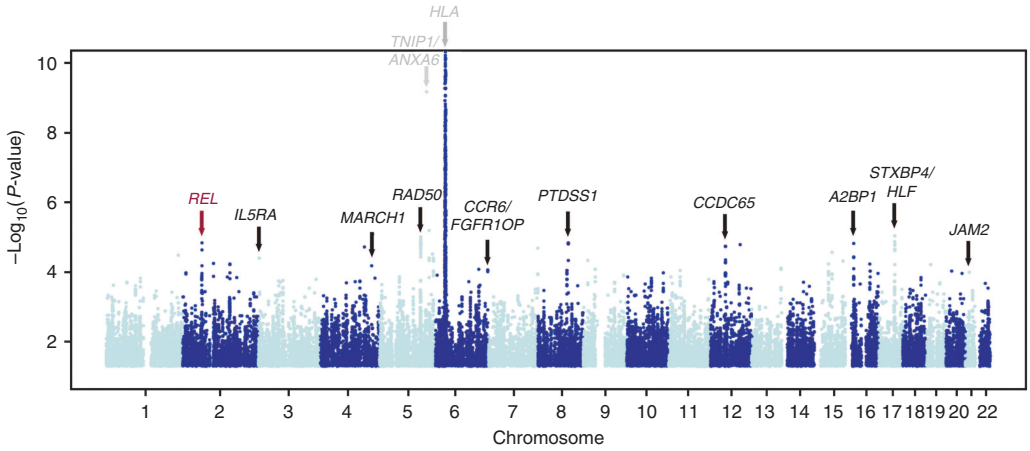

Figure 1. Results of genome-wide meta-analysis. The negative decadic logarithm of the corresponding *P*-value from the meta-analysis is shown for each single-nucleotide polymorphism (SNP), according to chromosome. All markers that passed quality control criteria, those that were available in all three genome-wide association studies (GWAS), and those that showed no heterogeneity between the studies were used for plotting. The plot was created with the software environment R version 2.11.1 (Team, 2007). The established PsA loci *HLA-C* and *TNIP1* are highlighted by gray arrows, whereas the follow-up loci are tagged by black arrows. The novel risk locus at *REL* is highlighted by a red arrow. The *HLA* region stands out clearly from all other loci. For better scaling, the *y* axis was limited to a maximum value of $-\log_{10}(P\text{-value}) = 10$, thereby truncating the *HLA* signal at this value.

5 kb downstream of *PUS10*—remained significantly associated with PsA, yielding $P = 4.24 \times 10^{-5}$ (OR = 1.23, 95% confidence interval (CI) = 1.14–1.33, no heterogeneity: $I^2 = 0$). In contrast, rs2829866 showed no evidence for association in the extended replication sample ($\alpha = 0.005$, calculated as 0.05/10). The combined analysis of the discovery and the six replication panels (2,466 PsA cases and 10,217 controls) yielded a genome-wide significant *P*-value of 1.18×10^{-8} (OR = 1.27, 95% CI = 1.18–1.35, $I^2 = 0$) for the lead SNP rs13017599 (Table 2). To fine map a region of 500 kb around the lead SNP rs13017599, we performed imputation based on CEU haplotypes generated by the 1000 Genomes Project for the three GWAS panels used for discovery phase (The 1000 Genomes Project Consortium, 2010). The regional plot of the combined *P*-values confirmed the results of the HapMap phase 3-imputed meta-analysis, with rs13017599 displaying the strongest association ($P = 1.88 \times 10^{-5}$) at this locus (Figure 2). In addition, we genotyped the SNP rs702873, which was reported to be associated with PsV by Strange *et al.* (2010) in the replication panels 1 through 6 (1,931 PsA cases and 6,785 controls). Meta-analysis of the three GWAS and the six replication study groups (2,466 PsA cases and 10,217 controls) yielded $P = 1.93 \times 10^{-7}$ (OR = 1.20, 95% CI = 1.13–1.28), confirming the newly identified association of this locus with PsA (Tables 1 and 2). The lead SNP rs13017599 and the PsV-associated variant rs702873 are about 83 kb apart and are in moderate linkage disequilibrium (linkage disequilibrium in 566 CEU haplotypes generated by the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2010); $r^2 = 0.625$). To test for the independence of these two markers, we performed a logistic regression analysis. When conditioning for the lead SNP rs13017599, rs702873 was not significantly associated with PsA ($P = 0.20$), whereas rs13017599 was still associated ($P = 2.90 \times 10^{-2}$) in the reverse conditioning, indicating that the two SNPs are not completely independent, but that rs13017599 accounts for the association of PsA at the *REL* gene locus rather than rs702873. To investigate whether the *REL* locus was more strongly associated with PsA than with PsV, we additionally analyzed the two *REL* SNPs rs13017599 and rs702873 in PsV cases without PsA from the same three GWAS and six replication panels (5,912 PsV cases without PsA and 10,217 controls). This analysis yielded much weaker association signals compared with the PsA cohort (rs13017599, $P_{PsV} = 3.48 \times 10^{-5}$, OR = 1.13, 95% CI = 1.08–1.19; rs702873, $P_{PsV} = 4.70 \times 10^{-6}$, OR = 1.14, 95% CI = 1.09–1.19), highlighting that the *REL* association is stronger in PsA, but nevertheless confirming the previously reported association of rs702873 with PsV. For each of the two associations at rs13017599 and rs702873, there is an overlap in CIs for PsA and PsV, which further proves that the *REL* gene represents a shared susceptibility locus for PsA and PsV.

DISCUSSION

In addition to the recently reported association of SNP rs13017599 with RA (Gregersen *et al.*, 2009) and SNP rs702873—located 27 kb upstream of the *REL* gene—with PsV (Strange *et al.*, 2010), several other SNPs downstream of *REL* have been identified to be associated with ulcerative colitis (McGovern *et al.*, 2010), Crohn's disease (Franke *et al.*, 2010), celiac disease (Dubois *et al.*, 2010), and primary sclerosing cholangitis (Janse *et al.*, 2011), demonstrating that *REL* is a shared disease locus of several complex diseases and thereby highlighting its role in inflammatory barrier disease susceptibility. *REL* encodes one of four subunits found in NF-κB dimers and belongs to the NF-κB (REL) family of transcription factors, which is highly important in the innate immune system. The above-mentioned association findings are therefore consistent with the importance of the NF-κB pathway in these inflammatory diseases. c-Rel contains trans-activation domains that are a prerequisite for gene

Table 1. Association results of ten follow-up SNPs from the meta-analysis and of the PsV-associated SNP rs702873

				Genome-wide analysis (535 cases, 3,432 controls)		Replication analysis (252 PsA cases, 1,740 controls)					Combined analysis (GWAS, Repl. 1+2) (787 PsA cases, 5,172 controls)	
						Allele frequencies (ca/co)		Repl. 1+2				
Locus	SNP	Chromosome position (bp)	Alleles	Meta *P*-value	OR (95% CI)	Repl. 1 Germany	Repl. 2 Estonia 1	P_{CMH}	OR (95% CI)	P_{BD}	Meta *P*-value	OR
STXBP4/HLF	rs171511	17 53,273,910	T/C	9.30×10^{-6}	1.37 (1.19–1.58)	0.39/0.36	0.42/0.44	0.59	1.06 (0.87–1.29)	0.33	8.62×10^{-5}	1.26
RAD50	rs6596086	5 131,952,222	T/C	9.77×10^{-6}	1.51 (1.26–1.81)	0.77/0.75	0.71/0.67	0.26	1.14 (0.91–1.42)	0.77	3.52×10^{-5}	1.35
REL/PUS10	rs13017599	2 61,164,331	G/A	1.48×10^{-5}	1.37 (1.19–1.57)	0.69/0.62	0.67/0.63	**8.92×10^{-3}**	1.31 (1.07–1.61)	0.63	4.56×10^{-7}	1.35
PTDSS1	rs7824505	8 97,330,691	A/C	1.49×10^{-5}	1.37 (1.19–1.57)	0.49/0.47	0.39/0.48	0.48	0.93 (0.77–1.13)	0.04	2.00×10^{-3}	1.20
CCDC65	rs10783293	12 49,308,475	A/G	1.83×10^{-5}	1.34 (1.17–1.53)	0.43/0.44	0.43/0.43	0.68	0.96 (0.79–1.17)	0.80	9.95×10^{-4}	1.20
A2BP1	rs12445208	16 7,325,638	A/C	3.72×10^{-5}	1.48 (1.23–1.78)	0.80/0.81	0.83/0.81	0.99	1.00 (0.78–1.28)	0.51	1.10×10^{-3}	1.28
IL5RA	rs3792424	3 3,117,700	A/G	3.97×10^{-5}	1.94 (1.42–2.67)	0.91/0.93	0.93/0.91	0.99	1.00 (0.69–1.43)	0.18	2.98×10^{-3}	1.43
MARCH1	rs7686154	4 164,542,625	T/G	6.56×10^{-5}	2.83 (1.70–4.71)	0.96/0.96	0.92/0.93	0.95	0.99 (0.63–1.54)	0.53	1.08×10^{-2}	1.55
CCR6/FGFR1OP	rs162295	6 167,454,097	A/C	8.62×10^{-5}	1.36 (1.17–1.59)	0.72/0.73	0.70/0.67	0.88	1.02 (0.82–1.26)	0.37	1.14×10^{-3}	1.23
JAM2	rs2829866	21 27,059,071	A/T	1.00×10^{-4}	1.32 (1.15–1.52)	0.37/0.30	0.27/0.25	**3.75×10^{-2}**	1.24 (1.01–1.53)	0.39	1.14×10^{-5}	1.30
PAPOLG/REL	rs702873	2 61,081,542	C/T	1.56×10^{-4}	1.29 (1.13–1.48)	0.61/0.56	0.63/0.57	**3.38×10^{-2}**	1.24 (1.02–1.50)	0.77	1.57×10^{-5}	1.28

Abbreviations: CI, confidence interval; CMH, Cochran–Mantel–Haenszel test; GWAS, genome-wide association study; OR, odds ratio; P_{BD}, asymptotic P_{BD}-value of the Breslow–Day test for heterogeneity; PsA, psoriatic arthritis; PsV, psoriasis vulgaris; Repl, replication; SNP, single-nucleotide polymorphism. The top 10 SNPs that were selected for follow-up from the meta-analysis are listed. These SNPs were genotyped in the replication case–control panel 1 and 2 from Germany and Estonia, respectively. Moreover, we genotyped the SNP rs702873 that was previously reported to be associated with PsV (Strange *et al.*, 2010). SNPs are ranked according to their *P*-values obtained in the initial meta-analysis. Positions (Position (bp)) are in NCBI's build 37. A significant P_{BD}-value indicates a significant heterogeneity between the two replication panels in terms of the odds ratio of the disease association. Combined *P*-values (P_{CMH}) and combined ORs of the CMH test statistic (one degree of freedom) are shown for replication panels 1 and 2. Significant *P*-values ($P_{CMH} < 0.05$ (only if $P_{BD} > 0.05$)) of the replication panels are highlighted in bold.

transcription, and T-cell–mediated immune responses were markedly impaired in c-Rel$^{-/-}$ animals. The confirmed PsA/PsV susceptibility locus *TNFAIP3* and a variety of other genes are regulated by c-Rel in T cells (Bunting *et al.*, 2007). Notably, c-REL mediates differentiation of CD4+ FoxP3+ regulatory T cells, which have the capacity to develop into IL-17-producing cells in psoriasis (Bovenschen *et al.*, 2011). It also promotes IL-12B and IL-23 expression, by which TH-1 and TH-17 types of immune response might be evoked (Mise-Omata *et al.*, 2007; Reinhard *et al.*, 2011). Altogether, these findings further support the hypothesis that *REL* is an important integrator of inflammatory signaling pathways observed in the complex emerging landscape of genetic susceptibility for PsA.

In conclusion, we were able to provide—to our knowledge previously unreported—genome-wide significant evidence for involvement of the *REL* locus in PsA susceptibility, and we could confirm the previously reported association with PsV. The current results demand not only a thorough analysis of all genes involved in this pathway to search for additional risk alleles but also overlap analyses between complex disorders sharing a certain genetic background, such as PsA, PsV, and RA. It should be noted, however, that because most of the patients develop PsA following the onset of PsV, PsA might be considered as a disease within a disease with psoriasis as the parent disease (Eder *et al.*, 2011), so that any association study on PsA is bound to identify PsV susceptibility genes such as the established PsV/PsA risk loci *HLA-Cw6*, *IL12B*, *IL23R*, *TNIP1*, and *FBXL19*, which do not allow for genetic discrimination between the two conditions. On the other hand, the genetic component of PsA is much higher than that of PsV, and only about one-third of PsV patients additionally develop PsA, implying that differences in the genetic backgrounds do exist. Genes involved in innate (such as *REL*) and adaptive immunity are expectedly more often shared between different immune-mediated diseases, whereas genes of skin barrier function are rather restricted to PsV. So far, no PsA-specific associations have been identified with genome-wide significance. Lack of power to find genetic variants with weaker effects, and rare variants that contribute

Table 2. Association results of the two follow-up SNPs and the PsV-associated *REL* SNP in replication panels 1 through 6

				Genome-wide analysis (535 cases, 3,432 controls)		Replication analysis (1,931 cases, 6,785 controls)										Meta-analysis (2,466 cases, 10,217 controls)	
						Allele frequencies (cases/controls)						Repl. 1+2		Repl. 1–6			
Locus	SNP	Chromosome position (bp)	Alleles	Meta *P*-value	OR (95% CI)	Repl. 1 Germany	Repl. 2 Estonia 1	Repl. 3 Michigan	Repl. 4 NPF	Repl. 5 CASP-DFU	Repl. 6 Estonia 2	P_{CMH}[1]	OR (95% CI)	Meta *P*-value	OR (95% CI)	Meta *P*-value	OR (95% CI)
REL	rs13017599	2 61,164,331	G/A	1.48×10^{-5}	1.37 (1.19–1.57)	0.69/0.62	0.67/0.63	0.69/0.65	0.71/0.71	0.69/0.64	0.70/0.64	8.92×10^{-3}	1.31 (1.07–1.61)	4.24×10^{-5}	1.23 (1.14–1.33)	1.18×10^{-8}	1.27 (1.18–1.35)
REL	rs702873	2 61,081,542	C/T	1.56×10^{-4}	1.29 (1.13–1.48)	0.61/0.56	0.63/0.57	0.62/0.57	0.61/0.62	0.61/0.56	0.64/0.58	3.38×10^{-2}	1.24 (1.02–1.50)	1.25×10^{-4}	1.17 (1.09–1.26)	1.93×10^{-7}	1.20 (1.13–1.28)
JAM2	rs2829866	21 27,059,071	A/T	5.73×10^{-5}	1.34 (1.16–1.54)	0.37/0.30	0.27/0.25	0.32/0.31	0.35/0.30	0.32/0.32	0.21/0.26	3.75×10^{-2}	1.24 (1.01–1.53)	0.11	1.07 (0.98–1.16)	6.33×10^{-4}	1.13 (1.05–1.21)

Abbreviations: CASP-DFU, CASP Deep Follow-up; CI, confidence interval; CMH, Cochran–Mantel–Haenszel test; GWAS, genome-wide association study; NPF, National Psoriasis Foundation; OR, odds ratio; P_{BD}, asymptotic *P*-value of the Breslow–Day test for heterogeneity; PsA, psoriatic arthritis; PsV, psoriasis vulgaris; Repl, replication; SNP, single-nucleotide polymorphism.
[1]Cochran–Mantel–Haenszel test; Breslow–Day test, P=0.63 for rs13017599, P=0.77 for rs702873 and P=0.39 for rs2829866.
We analyzed the top 10 SNPs of the GWAS meta analysis (including typed and imputed genotypes) in two independent PsA case-control panels from Germany and Estonia (Repl. 1 and 2, respectively). The two SNPs with nominal significant replication results ($P < 0.05$) in these two replication panels as well as the PsV-associated SNP rs702873 were genotyped in four additional independent PsA case–control panels (Repl. 3 through 6). Results of all 10 SNPs are shown in Table 1. Allele frequencies are given for each of the six replication panels separately. Nucleotide positions refer to NCBI build 37.

to disease risk, might explain the sparse results, thereby emphasizing the need for larger studies and targeted next-generation sequencing of candidate regions.

MATERIALS AND METHODS

Subjects

All German PsA (219) and PsV (733) cases in the GWAS panel and in the replication panel 1 were recruited either at the Department of Dermatology of the Christian-Albrechts-University Kiel or at the Department of Dermatology and Allergy of the Technical University Munich through local outpatient services. PsA was diagnosed by a clinical finding of joint complaints and radiological and rheumatological confirmation of criteria according to Moll and Wright (1973), or, more recently, to the Classification Criteria for Psoriatic Arthritis (CASPAR; Taylor *et al.*, 2006). In all, 63 PsA cases from Munich were diagnosed by a physician, with negative diagnosis of RA. Individuals were considered to be affected by PsV if chronic plaque or guttate psoriasis lesions covered more than 1% of the total body surface area or if at least two skin, scalp, nail, or joint lesions were clinically diagnosed as characteristic of psoriasis by a dermatologist.

In all, 2,127 German healthy control individuals in the GWAS panel and the replication panel 1 were obtained from the PopGen biobank (Krawczak *et al.*, 2006). A total of 465 German healthy controls were selected from the KORA S4 survey, an independent population-based sample from the general population living in the region of Augsburg, southern Germany (Wichmann *et al.*, 2005).

The CASP GWAS (for details see (Nair *et al.*, 2009; Stuart *et al.*, 2010)) consisted of 335 PsA cases, 915 PsV cases, and 1,322 controls after quality control measures. The data sets used for the analyses described in this manuscript were obtained from the database of Genotype and Phenotype (http://www.ncbi.nlm.nih.gov/gap).

The Canadian GWAS sample (from Genizon BioSciences, St Laurent, Quebec, Canada) consisted of 139 PsA cases, 614 PsV cases, and 987 controls sampled from the Québec founder population. Membership in the Québec founder population was defined as having four grandparents with French–Canadian family names who were born in the Province of Québec, Canada, or in adjacent areas in the provinces of New Brunswick and Ontario, or in New England or New York State. This criterion assured that all subjects were descendants of French Canadians living before the 1960s, after which time admixture with non-French Canadians became more common. Inclusion criteria were the presence of plaque-forming psoriasis determined by a dermatologist, and disease onset between 18 and 40 years of age. Exclusion criteria were other forms of psoriasis, such as pustular, guttate, inverse, erythrodermic, and isolated site psoriasis and late onset. The presence of PsA was noted, but was not an ascertainment criterion.

The samples of the Estonian replication panel 2 were unrelated Estonian Caucasian patients with a clear clinical diagnosis of PsV, collected at the Department of Dermatology and Venerology and at the Department of Physiology and Centre of Translational Medicine at the University of Tartu. Patients were classified as having PsA if this diagnosis had been established by an experienced rheumatologist. The Estonian replication panel 6 consisted of samples provided by the population-based biobank of the Estonian Genome Center, University of Tartu. Subjects were recruited by general practitioners and physicians in the hospitals. Physicians in the hospitals were randomly selected from individuals visiting general practitioner

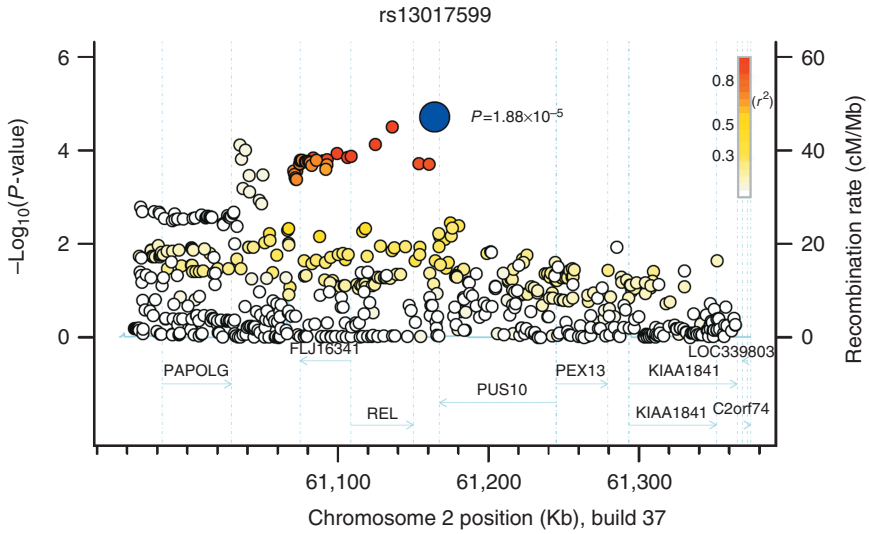

Figure 2. Regional plot of the *REL* locus. Regional plot of the negative decadic logarithm of the combined *P*-values from the imputed meta-analysis of three genome-wide association studies (GWAS). A window of 500 kb around the lead single-nucleotide polymorphism (SNP) rs13017599 (blue filled circle) is shown. The three GWAS panels were imputed with CEU haplotypes generated by the 1,000 Genomes Project (August 2010 release) as a reference. The magnitude of linkage disequilibrium with the central SNP rs13017599, measured by r^2, is reflected by the color of each SNP symbol (color coding: see upper right corner of the plot).

offices or hospitals. Diagnosis of PsV and/or PsA based on clinical symptoms was posed by a general practitioner and confirmed by a dermatologist. At the time of recruitment, the controls did not report diagnosis of osteoarthritis, psoriasis, or autoimmune diseases.

Replication panels 3 through 5 (Michigan, National Psoriasis Foundation, and CASP-DFU) consisted of 1,649 PsA cases, 2,349 PsV cases, and 4,061 controls of white European ancestry from the United States and Canada. Diagnosis of psoriasis was always confirmed by a dermatologist. All PsA patients were diagnosed by a rheumatologist and/or fulfilled CASPAR criteria for the classification of PsA. All Candadian PsV patients underwent a rheumatological examination to rule out the presence of inflammatory arthritis. Patients with other forms of inflammatory arthritis were excluded.

Written, informed consent was obtained from all study participants, and all protocols were approved by the respective institutional ethics review committees of the participating centers. The investigations were conducted according to the Declaration of Helsinki Principles.

The samples were organized in panels, which corresponded to the successive steps of the present study, and all panels (GWAS panels and replication panels 1 through 6) were independent from each other.

Genotyping

The genotyping for the German GWAS, which was part of the German GWAS initiative funded by the National Genome Research Network, was performed by Illumina's service facility using the Illumina HumanHap 550K v1 with 561,466 SNP markers (Illumina, San Diego, CA). All experimental steps were carried out according to the standard protocols. For genome-wide genotyping of the CASP samples, the Perlegen 600k array was used (Perlegen Sciences, Mountain View, CA). The genotyping was provided through the Genetic Association Information Network. The Illumina Human 1M BeadChip was used for generating the Canadian genome-wide data (Genizon GWAS), as well as for genotypes of SNPs rs13017599 and rs2829866 in replication panel 5 (CASP-DFU). Functionally tested TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) were used to genotype variants in replication panels 1 through 6.

Statistical analyses

We used GWAS data sets that passed stringent quality control filters for genotype imputation with Beagle (Browning and Browning, 2009) using HapMap phase 3 reference samples from the CEU, TSI, MEX, and GJT collections, as well as for the subsequent statistical analyses. Low genomic-control values for all three scans (German GWAS: $\lambda_{GC} = 1.01$, CASP GWAS: $\lambda_{GC} = 1.02$, and Canadian GWAS: $\lambda_{GC} = 1.03$) indicated a minimal overall inflation of the test statistics due to population stratification.

Power calculations were carried out using the PS Power and Sample Size v3.0.12 (Dupont and Plummer, 1997; Supplementary Figure S2 online). GWAS data were analyzed using R statistical environment version 2.10.0 and gPLINK v2.049 in combination with PLINK v1.05 (Purcell *et al.*, 2007).

We selected 10 SNPs based on their *P*-value ranking for replication in two independent samples from Germany and Estonia comprising 252 PsA cases and 1,740 controls. All SNPs passed quality control measures, which means that they had a high call rate (>95% in cases or controls), were not monomorphic (minor allele frequency >1% in cases or controls), and did not deviate from HWE in the control population (exact HWE $P > 10^{-4}$). Two of the 10 follow-up SNPs were significantly associated with PsA and were therefore genotyped in four additional replication panels from the United States, Canada, and Estonia.

The Cochran–Mantel–Haenszel test was used for combined replication analysis of replication panel 1 and 2 from Germany and Estonia, to take the possibility of minor differing genetic backgrounds into account. The initial GWAS meta-analysis was performed using PLINK's meta-analysis function with its standard

error of OR weighting option. Association results of replication panel 5 (CASP-DFU) were obtained with the statistical association test ''Efficient Mixed-Model Association eXpedited'' to account for sample structure (Kang *et al.*, 2010), and the combined replication analysis of all six replication panels, as well as the combined GWAS-replication analysis, was performed with METAL (Willer *et al.*, 2010). Logistic regression analysis to test for the independence of the two *REL* SNPs rs13017599 and rs702873 was performed with PLINK using the conditional analysis commands *logistic* and *condition* to test one SNP, but adding the allelic dosage for the other SNP as a covariate. The conditional analysis was carried out for each cohort separately and results were subsequently combined by means of meta-analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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